Development of Alternative *in vitro* Assays for the Prioritization and Screening of Chemicals for the U.S. EPA's Endocrine-Disruptor Screening Program

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The U.S. EPA is currently implementing an Endocrine Disruptor Screening Program (EDSP) designed to detect chemicals that alter the estrogen, androgen, and thyroid systems in human, fish, and wildlife. To date, the focus has been the development of a priority-setting approach to establish which chemicals will be initially tested (www.epa.gov/scipoly/oscpendo/prioritysetting/overview.htm) and the development, standardization, and validation of assays for use in the Tier I Screening (TIS) and Tier 2 Testing Batteries (www.epa.gov/scipoly/oscpendo/assayvalidation/index.htm). While NHEERL scientists have played an integral role in the development of the assays in the TIS Battery, we are continuing to develop alternative in vitro approaches that should provide a new tool for priority setting and improved assays for a "2nd Generation" TIS Battery. Here, we focus upon two studies that are currently part of the Agency's Computational Toxicology Program. The first study is generating receptor-binding data to provide a well-defined data set of approximately 300 chemicals that will serve as a training set for the development of Quantitative Structure Activity Relationship (QSAR) models designed to detect chemicals that are likely to bind to the estrogen receptor (ER). These results will not only allow the establishment of performance criteria that must be applied when interpreting any type of receptor-binding data, but they will also provide a set of ER binding data for a group of structurally diverse chemicals selected from within the Agency's chemical universe. Moreover, only chemicals that demonstrate true competitive binding as determined by actual Ki experiments will be designated as binders. It is imperative that such data be provided, as the Agency's previous evaluation of two ER QSAR models has demonstrated that earlier models may not have been based upon chemicals demonstrating true competitive inhibition and that such a data set is essential to improve the sensitivity, specificity, and positive predictive probability of these QSAR models. A second focus of our research involves the development of a test system to assess the effects of potential endocrine disruptors on key steps in steroid synthesis. For this purpose, a human H295R adrenocortical tumor cell line is being employed that retains the ability to produce a multitude of steroids within this synthetic pathway: progestins, androgens, estrogens, and gluco- and mineralocorticoids. Expression levels of mRNA throughout the steps in steroid synthesis will be measured using a quantitative, real-time, reverse transcriptase polymerase chain reaction (QT-RT-PCR) method that should allow determination of a site within the pathway being targeted by an endocrine-disrupting compound. The H295R cell protocol offers several advantages over the current T1S approaches using sliced rat testis (for measurement of testosterone) and placental or recombinant aromatase assays (for the measurement of estrogen). Most importantly, this assay provides the means to examine the production of all steroids of interest at once and would eliminate the use of animals for such determinations.